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The use of muscle enzymes as predictors of pork meat quality

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Abstract

Muscle endo-protease (calpains and cathepsins) and exo-protease (dipeptidyl-peptidases and aminopeptidases) activities were assayed at 2 h post-mortem in different meat qualities (PSE, RSE, RFN and DFD). The sensory characteristics of the different pork meat qualities were also evaluated in order to correlate them to the proteolytic activity. The assay of aminopeptidase and dipeptidylpeptidase activities (AAP, RAP, LAP, DPPI and DPPIV) at 2 h post-mortem discriminate between exudative and non-exudative classes explaining 74.6% of the variability. Also, at 24 h post-mortem 71.2% of the variability was detected by the measurement of PGAP, AAP, RAP, DPPII and DPPIV. Therefore, the exoprotease activities can constitute a novel and adequate technique to predict early post-mortem pork meat quality allowing its assay till 24 h post-mortem because of the good stability of the enzymes during this post-mortem time.

Keywords: Muscle enzymes; Proteases; Exopeptidases; Pork; Meat quality

1. Introduction

The prediction of pork quality on the slaughter line is not an easy task because some of the biochemical quality properties have not enough time to be fully developed. The evaluation of pork quality should be based on relatively inexpensive and rapid measurements taken in the slaughterline where carcass identification is available in order to have better use of the product for further processing and distribution. There are many techniques such as pH, colour, electrical conductivity and drip loss (DL) currently used for carcass classification. Although they have to be applied at specific post-mortem times, 2 and 24 h, or even require time, 4 days of storage in the case of DL for a correct prediction of the quality. Thus, there is a need to find new biochemical assays, well correlated with carcass quality, in order to have a proper classification in a shorter time.

During the last decades many methods have been developed to detect exudative meats. Several methods have been optimised at pre-rigor state such as the measurements of pH_{45min} (Somers, Tarrant & Sherington, 1985) that is extensively used in slaughterhouse although presents certain variability. Other methods consist in the measurement of reflectance (Chizzolini, Novelli, Badiani, Delbomo & Rosa, 1993; Warris & Brown, 1987), electrical conductivity (Garrido, Pedauyé, Bañón, Lopez & Laencina, 1995) and impedance (Swatland, 1995). At post-rigor state, the measurement of water holding capacity (Warris & Brown, 1987) and the measurements of luminosity (Garrido, Pedauyé, Bañón & Laencina, 1994; Laack, Kauffman, Sybesma, Smulders, Eikelenboom & Pinheiro, 1994) were developed. However, the presence of intermediate qualities among the extremes PSE and DFD increase the variability. This is the case of the high incidence of RSE (reddish-pink, soft and exudative) meat that has an economic impact in the pig industry (Cheah, Cheah & Just, 1998). RSE meat is characterised by an exceptionally high drip loss with normal red colour (Kauffman, Cassens, Cherer & Meeker, 1992; Laack et al., 1994; Warner, Kauffman & Russell, 1997). This meat might be due to a genetic disposition or also be induced by poor handling (Cheah et al., 1998). From the processor point of view, it is important to be able to predict the water holding capacity of meat (WHC) because it is responsible for weight loss in raw, cooked and processed meats. It is also responsible of poor colour in cured meat products, such as ham, and can influence meat palatability traits. So, recent reports have been focused in the study of new techniques for a better prediction of meat quality (Boland et al., 1995; Garrido & Honikel, 1996; Kauffman et al., 1993).

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On the other hand, there is a great interest in the study of biochemical characteristics and genetic factors that can contribute to meat quality, not only in the carcass but also on the live animal being of great importance to animal production. The use of genetic technology is playing an important role in improving meat quality (Tarrant, 1998). Among biochemical characteristics, the enzymes, citrate synthase and lactate dehydrogenase indicative of the aerobic and anaerobic capacity, respectively, have been poorly related to meat quality (Henckel, Oksbjerg, Erlandsen, Barton-Gade & Bejerholm, 1997). Other studies, have been focused in the study of the muscle glycolytic potential to predict quality (Maribo, Stoier & Jorgensen, 1999; Monin & Sellier, 1985) and in the origin of PSE syndrome based on the enzymatic activities, pyruvate kinase (Schwägele, Haschke, Honikel & Krauss, 1996) and glycogen phosphorilase (Schwägele, Lopez Buesa & Honikel, 1996).

Other group of well-known muscle enzymes are proteases. The proteolysis in the post-mortem muscle contribute to the development of meat flavour and therefore to the eating quality of meat (Wood et al., 1996). First, the action of endopeptidase will affect the texture of the meat, being the rate of tenderisation affected by the post-mortem pH because calpain and lysosomal cathepsin activities are dependent of pH (Beltran, Jaime, Santolaria, Sañudo, Alberti & Roncalés, 1997; Watanabe, Daly & Devine, 1996). However, tenderness is also depending on the shortening of the muscle (Koohmaraie, 1996). Second, exopeptidases act over polypeptides and proteins generating smaller peptides and free amino acids responsible of flavour changes in meat and meat products (Aristoy & Toldrá, 1995; Kato, Rhue & Nishimura, 1989; Nishimura, Rhue, Okitani & Kato, 1988). Therefore, the study of the proteolytical system in the different pork qualities can be useful not only to find parameters that can predict technological pork quality but also to detect differences on sensory quality of pork meat.

The objective of this study is to develop a new methodology based on enzymatic measurements that will be able to predict meat quality at early post-mortem.

2. Materials and methods

2.1. Animals

Carcasses were obtained from commercial slaughterhouses closely located to our Institute. 59 pork carcasses (mother: Large White×Landrace, father: Large White) from 6-month old pigs, representing a broad array of pork quality, were selected. The muscle *Longissimus dorsi* was removed at 2 h post-mortem. Two slices were inmediately sampled at 2 and 24 h of postmortem storage for enzyme analysis. After 4 days of postmortem storage at 4°C, two slices from each loin were removed, vacuum packaged and kept under frozen storage at −80°C till sensory analysis.

2.2. Meat quality measurements

The measurements were made on the right hand side of the carcass. The pH was measured at 2 and 24 h in the muscle *Longissimus dorsi* at the level of the fifth rib with a portable pH-meter Hanna HI 8424 (Hanna Instruments, Portugal). The colour, L^*, a^*, b^* coordinates, were measured at 24 h post-mortem with a Hunter labscan Chromameter (Hunter, Reston, VA). The drip loss (DL) was measured by the method of Warris (1982). The intramuscular fat content (IMF) was analysed by following the method of Folch, Lees & Stanley (1957). Moisture content was determined after dehydration at 100°C to a constant weight (ISO, 1973) and protein content was analysed using the Kjeldahl method (AOAC, 1990).

2.3. Preparation of enzyme extracts for calpain/calpastatin assays

Ten g of muscle was homogenised in 30 ml of 50 mM Tris buffer containing 3 mM EDTA and 10 mM mercaptoethanol, pH 8.5. The extract was homogenised (3×10 s at 27 000 rpm with cooling on ice) with a polytron (Kinematica, Switzerland), centrifuged at 27 000 *g* for 20 min at 4°C and the supernatant filtered through glass wool and adjusted to pH 7.5 and centrifuged again in the same conditions. The supernatant was used for calpain/calpastatin assays.

2.4. Preparation of enzyme extracts for cathepsin/peptidases assays

Four g of muscle was homogenised in 20 ml of 50 mM sodium citrate buffer containing 1 mM EDTA and 0.2% (v/v) Triton X-100 at pH 5.0 (case of cathepsins) or 20 ml of 50 mM disodium phosphate buffer, pH 7.5, containing 5mM EGTA (case of peptidases). In both cases, the extract was homogenised (3×10 s at 27 000 rpm with cooling on ice) with a polytron (Kinematica, Switzerland), centrifuged at 12 000 *g* for 20 min at 4°C and the supernatant filtered through glass wool and used for further enzyme assays.

2.5. Assay of enzyme activities

Calpains I and II and calpastatin were assayed after FPLC purification with a DEAE column by using casein-fluorescein isothiocyanate (FITC) (Sigma, St Louis, MO) as substrate (Rosell & Toldrá, 1996). Cathepsins

were assayed as described by Toldrá and Etherington (1988) using *N*-CBZ-L-arginyl-7-AMC, *N*-CBZ-L-phenylalanyl-L-arginine-7-AMC, both at pH 6.0, and L-arginine-7-AMC (Sigma, St Louis, MO) for cathepsins B, B + L and H, respectively. Dipeptidyl peptidases (DPP) I, II, and IV were assayed as previously described by Blanchard, Ellis, Maltin, Falkous, Harris and Mantle (1993), using H-Glycyl-Arginyl-AMC (Bachem, Switzerland), lysyl-alanyl-AMC (Sigma, St Louis, MO), and glycyl-prolyl-AMC (Sigma, St Louis, MO), as respective fluorescent substrates. Alanyl (AAP), arginyl (RAP), leucyl (LAP), pyroglutamyl (PGAP) aminopeptidases were assayed as previously described by Flores, Aristoy and Toldrá (1997) using the fluorescent substrates arginyl-AMC, alanyl-AMC, leucyl-AMC and pyroglutamyl-AMC (Sigma, St Louis, MO), respectively. In all cases, the reaction was incubated at 37°C and the fluorescence continuously monitored at 355 and 460 nm for AMC derivatives and 485 and 538 nm for FITC derivatives, as excitation and emission wavelengths, respectively, using a Fluoroskan II multiscanning fluorimeter (Labsystems, Helsinki, Finland). Four replicates were performed for each enzyme assay. One unit (U) of proteolytic activity was defined as the amount of enzyme capable of hydrolysing 1 μmol of substrate per hour at 37°C.

2.6. Sample preparation for cooked pork loin

The samples were thawed at room temperature during 4 h in a plastic vacuum bag and prepared by inmersion in a water bath at 70°C for 1 h. Then, the samples were cut into 1 cm cubes and immediately presented to the panel.

2.7. Sensory analysis of pork loin

Samples were evaluated for sensory quality using quantitative descriptive analysis (QDA) (Stone, Sidel, Oliver, Woolsey & Singleton, 1974). Eight panelists were selected among the personnel of the institute. The training of the panel and the development of an specific descriptive lexicon for pork loin was done as previously described by Flores, Armero, Aristoy and Toldrá (1999). The study consisted in 10 sessions, with six samples analysed per session. Each sample was presented (two cubes per sample) in a petri-dish at 5 min intervals. The panel was instructed to open the petri-dish, smell the aromatics in three short sniffs, and place one of the cubes into the mouth for "flavour-by-mouth" assessment. Approximately 2 min elapsed before evaluating the second cube. The highest intensity for an attribute of the two cubes was recorded. In order to rinse the mouth between samples, water and unsalted toasted bread were used. Intensities were based on unequal-interval scale (Stone et al., 1974) and recorded via computer ballot system.

2.8. Statistical methods

The analysis of variance (ANOVA) was used to analyse the enzymatic and sensory data in the different qualities using the statistical package Statgraphic plus (v 2.0). The Fisher's least significant difference (LSD) procedure was used to discriminate among the means of sensory descriptors and enzyme activities in the different post-mortem meat quality groups. Stepwise discriminant analysis (BMDP package) using as variables enzymes at 2 or 24 h was used to reclassify the samples into previously defined groups according to pH_{2h}, pH_{24h}, L and DL values a usual grouping procedure in processing plants. Principal component analysis (PCA) was performed using Statgraphics plus (v 2.0).

3. Results and discussion

Pork quality must be optimised and consistently presented to consumer and meat processor in order to insure continued acceptance. Therefore, in slaughterhouses is a priority the evaluation of the pork carcasses in a shorter time. Actually, the pH, colour and drip loss (DL) are used to classify the different pork meat qualities (Kauffman et al., 1993; Warner, Kauffman & Russell, 1993, 1997). So, in our study the carcasses were classified based upon pH, L, and DL into one of the following categories (Garrido & Honikel, 1996; Warner et al., 1993, 1997) described as follows:

Pale soft exudative (PSE)	pH_{2h} < 5.8	–	L > 50	DL > 6%
Red soft exudative (RSE)	pH_{2h} < 5.8	–	L = 44–50	DL > 6%
Red firm non-exudative (RFN)	pH_{2h} > 5.8	pH_{24h} < 6.0	L = 44–50	DL < 6%
Dark firm dry (DFD)	–	pH_{24h} > 6.0	L < 44	DL < 3%

3.1. Enzyme activity

Based on this classification, the proteolytic activities in postmortem muscle in the different quality classes were determined. The activity of the calpain system at 2 h postmortem is shown in Table 1. The μ-calpain and m-calpain activities were significantly higher ($P < 0.05$) in the DFD class than in the other classes. PSE and RSE classes were not different in the content of μ-calpain from the RFN class. On the other hand, no significant ($P < 0.05$) difference was found for the inhibitor

Table 1
Comparison of calpain system activity (expressed as U/g meat) assayed at 2 h post-mortem in different pork quality classes[a]

	PSE ($n=22$)		RSE ($n=7$)		RFN ($n=20$)		DFD ($n=10$)	
	M	SE	M	SE	M	SE	M	SE
μ-Calpain	0.057b	0.004	0.056b	0.008	0.058b	0.005	0.083a	0.006
m-Calpain	0.149b	0.006	0.142b	0.011	0.149b	0.006	0.175a	0.009
Calpastatin	0.020	0.002	0.023	0.004	0.021	0.002	0.031	0.004

[a] Means in a row with different letters are significantly different ($P<0.05$).

calpastatin among the four quality classes. The activity of μ-calpain in post-mortem muscle is influenced by several factors; ultimate pH, rate of pH decay, initial calpastatin level, temperature fall and the inactivation of μ-calpain through autolysis or denaturation (Geesink et al., 1992). It is also known that a high pH enhances the activity of μ- and m-calpain. Koohmaraie (1992) reported that the autolysis of μ-calpain is increased when the pH decreases from 7 to final post-mortem pH around 5.8. However, the rate of autolysis is decreased by lowering the temperature from 25 to 5°C. Therefore, a higher activity of μ- and m-calpain found in the DFD group should bring an enhanced proteolytic activity in muscles with a high ultimate pH. Beltrán et al. (1997) did not find any effect of high pH meat on protease activity at 2 h post-mortem but at 7 days post-mortem they showed a higher m-calpain activity for the DFD group. However, other authors have found a higher μ-calpain activity at 3 h post-mortem in fast glycolysing muscles than in slow ones (O'Halloran, Troy, Buckley & Reville, 1997). On the other hand, the activity of cathepsins did not present any difference among quality classes (data not shown). However, O'Halloran et al., 1997 reported an enhanced release of cathepsin B and L from lysosomes in fast glycolysing muscle.

Few reports deal with the contribution of muscle exoproteases in post-mortem meat quality. Dipeptidyl peptidases (DPP) generate dipeptides from the amino terminus of polypeptides and proteins. The in vivo role of dipeptidyl peptidases is related to peptide processing for different purposes such as regulation of bioactive peptide hormones and protein turnover in the normal cell cycle. However, its role during meat ageing and meat processing has not been fully established yet (Sentandreu & Toldrá, 1998). In our study, dipeptidyl peptidases presented several differences among pork qualities as shown in Table 2. The DFD quality class showed a significantly ($P<0.05$) lower DPP I activity than in PSE class, although at 24 h this difference disappeared. DPP II was significantly ($P<0.05$) higher in DFD than in the other classes and its activity was the lowest in RSE class, although there were no differences between PSE and RFN classes being these differences also detected at 24 h postmortem. DPP IV was significantly ($P<0.05$) lower in PSE than in RFN and DFD classes, being also DPP IV activity significantly ($P<0.05$) lower in RSE class than in DFD class. The lower DPPIV activity found in PSE class was also detected at 24 h post-mortem. There are no reports of dipeptidyl peptidase activity in different pork meat qualities but only in different types of pigs (Rosell & Toldrá, 1998) where the activity of DPP II and IV was found to be higher in white pigs than in Iberian pigs.

Aminopeptidases are the enzymes involved in the last step of the proteolytic chain. These enzymes contribute to taste development during meat ageing (Nishimura et al., 1988) and meat products (Flores, Sanz, Spanier, Aristoy & Toldrá, 1998; Toldrá & Flores, 1998; Toldrá,

Table 2
Comparison of dipeptidyl peptidase activity (expressed as U/g meat) assayed at 2 and 24 h post-mortem in different pork quality classes[a]

	PSE ($n=22$)		RSE ($n=7$)		RFN ($n=20$)		DFD ($n=10$)	
	M	SE	M	SE	M	SE	M	SE
2 h post-mortem								
DPPI	0.43a	0.02	0.36ab	0.04	0.37ab	0.02	0.29b	0.03
DPPII	0.11b	0.005	0.09c	0.008	0.12b	0.005	0.15a	0.006
DPPIV	0.93c	0.03	0.94bc	0.05	1.05ab	0.03	1.13a	0.04
24 h post-mortem								
DPPI	0.49	0.04	0.36	0.07	0.50	0.04	0.40	0.06
DPPII	0.08bc	0.004	0.07c	0.007	0.09b	0.004	0.11a	0.006
DPPIV	0.75b	0.04	0.83ab	0.06	0.92a	0.04	0.95a	0.05

[a] Means in a row with different letters are significantly different ($P<0.05$).

Flores & Aristoy, 1995) by hydrolysing amino acids from the amino terminus of oligopeptides. The free amino acids are of great importance because of their specific tastes (Nishimura & Kato, 1988) and also for their involvement in further degradation reactions that generate volatile compounds (Shahidi, Rubin & D'Souza, 1986). Alanyl and arginyl aminopeptidases are the most important aminopeptidases in muscle and are characterised by their broad and basic substrate specificity, respectively (Flores, Aristoy & Toldrá, 1993, 1996). Other aminopeptidases found in skeletal muscle are leucyl and pyroglutamyl aminopeptidases, although they are present in low proportion in muscle (Toldrá et al., 1995). Therefore, the action of exopeptidases is very important for meat quality because these enzymes are involved in the post-mortem breakdown of protein and peptides to free amino acids. In our study, the main differences by quality classes were observed in aminopeptidase activities (Table 3). At 2 h post-mortem, AAP was significantly ($P < 0.05$) higher in RFN and DFD classes than in RSE and PSE. This difference was also detected at 24 h post-mortem. The RAP activity was significantly ($P < 0.05$) lower in RSE class than in DFD class. However, at 24 h the PSE class was the one that presented significantly difference ($P < 0.05$) from DFD class. PGAP activity was not significantly different ($P < 0.05$) at 2 h but at 24 h it was significantly lower ($P < 0.05$) in PSE than in the other classes. No differences were found in LAP activity among classes either at 2 or 24 h postmortem. In summary, the aminopeptidase activities were lower in the exudative meats (PSE and RSE), and therefore, the high activity found in RFN and DFD classes should promote a higher release of free amino acids. The activity of aminopeptidases has been studied in different cross-breeds (Armero, Baselga, Aristoy & Toldrá, 1999) and in light and heavy pigs (Rosell & Toldrá, 1998; Toldrá, Flores, Aristoy, Virgili & Parolari, 1996). Rosell and Toldrá (1998) found higher levels of alanyl aminopeptidase in Iberian pigs than in white pigs.

The technological parameters used for the classification of meat quality showed good correlations with enzyme activities (Table 4). pH_{24h} was correlated with μ-calpain, m-calpain, AAP, RAP, DPPII and DPPIV while the DL presented negative correlations with μ-calpain, AAP, RAP, DPPII and DPPIV. When the enzyme activities were measured at 24 h post-mortem the relations of pH_{24h} and DL with AAP and DPPIV were maintained (data not shown). In summary, the non-exudative and high ultimate pH pork meats have shown higher enzyme activities. Beltrán et al. (1997) reported a high correlation of pH at 7 days post-mortem with m-calpain in beef ($r = 0.824$). They concluded that high ultimate pH increased m-calpain activity and resulted in an enhanced tenderisation of beef meat.

The composition of meat is very important for meat quality (Wood et al., 1996). In our study, no significant

Table 4
Correlation coefficients among technological parameters and muscle enzyme activities at 2 h post-mortem

	pH_{2h}	pH_{24h}	L	DL
μ-Calpain	0.207	0.507[a]	−0.088	−0.355[a]
m-Calpain	0.012	0.387[a]	−0.069	−0.239
Calpastatin	0.206	0.084	−0.199	−0.158
AAP	0.251	0.461[a]	0.097	−0.425[a]
RAP	0.196	0.357[a]	0.259	−0.337[a]
PGAP	0.419[a]	0.068	−0.169	−0.178
LAP	−0.088	0.147	0.326[a]	−0.146
DPPI	−0.239	−0.252	0.182	0.335[a]
DPPII	0.221	0.651[a]	0.295[a]	−0.489[a]
DPPIV	0.417[a]	0.401[a]	0.085	−0.493[a]

[a] Statistical significant difference of the estimated correlations ($P < 0.05$).

Table 3
Comparison of aminopeptidase activity (expressed as U/g meat) assayed at 2 and 24 h post-mortem in different pork quality classes[a]

	PSE ($n = 22$)		RSE ($n = 7$)		RFN ($n = 20$)		DFD ($n = 10$)	
	M	SE	M	SE	M	SE	M	SE
2 h post-mortem								
AAP	8.66b	0.39	7.90b	0.66	9.84a	0.39	10.40a	0.56
RAP	0.64ab	0.02	0.58b	0.04	0.66ab	0.02	0.71a	0.03
PGAP	0.26	0.02	0.25	0.04	0.29	0.02	0.32	0.03
LAP	0.45	0.01	0.41	0.02	0.44	0.01	0.46	0.02
24 h post-mortem								
AAP	8.09b	0.37	7.47b	0.65	9.37a	0.39	10.08a	0.55
RAP	0.56b	0.02	0.60ab	0.04	0.60ab	0.02	0.65a	0.03
PGAP	0.14b	0.02	0.20ab	0.03	0.23a	0.02	0.26a	0.02
LAP	0.49	0.02	0.52	0.05	0.50	0.02	0.51	0.02

[a] Means in a row with different letters are significantly different ($P < 0.05$).

differences ($P < 0.05$) were detected in composition among classes (data not shown) and also few correlations ($P < 0.05$) were obtained between composition and enzyme activities. This is the case of m-calpain that was positively related with intramuscular fat ($r = -0.344$) and moisture ($r = 0.490$).

From the ANOVA results we have observed that several aminopeptidase and dipeptidyl peptidase activities were lower in the exudative groups (PSE and RSE) than in the non-exudative groups (RFN and DFD). Therefore, in order to see if the enzymes can be used as predictors of the exudative meats we did a stepwise discriminant analysis between these two groups using as variables the enzyme activities at 2 or 24 h. In the procedure, $DPPIV_{2h}$, $DPPI_{2h}$, AAP_{2h}, LAP_{2h} and RAP_{2h} were selected and explained 74.6% of the variability of meat quality. The same stepwise discriminant analysis was performed using the enzyme activities measured at 24 h post-mortem. In this case, the selected variables were $PGAP_{24h}$, AAP_{24h}, RAP_{24h}, $DPPII_{24h}$ and $DPPIV_{24h}$ that explained 71.2% of the variability. So, the differences in enzyme activities among classes, mainly in exoproteases, could be used as indicators or markers for post-mortem quality in a broad time range (from 2 to 24 h).

3.2. Sensory analysis

In order to study how the post-mortem pork quality affected the final sensory quality of meat, a lexicon for cooked pork loins was used as previously described (Flores et al., 1999). The mean values of the descriptors for cooked pork loin evaluated in the four different classes and by sex are shown in Table 5. From the aromatics, only "serum" presented differences by sex, being significantly ($P < 0.05$) lower in meat from male than from female. In tastes, the sour taste was significantly ($P < 0.05$) lower in pork meat from male than from female. The "umami" sensation was also significantly ($P < 0.05$) lower in DFD than in RSE class. The effect of post-mortem meat quality on taste has not been previously reported except a few works on flavour (Bennet, Bramblett, Aberle & Harrington, 1973; Göransson, von Seth & Tornberg, 1992) but without finding any significant effect. A few differences in taste, such as sour and salty taste that were lower in the DFD class than in the other classes among qualities have been reported (Flores et al., 1999).

The texture of cooked pork loin showed differences among quality classes. The juiciness was significantly ($P < 0.05$) lower in RSE than in RFN and DFD classes, although the PSE class did not differ from the other classes. The hardness did not show differences among classes although it was significantly ($P < 0.05$) higher in pork from female than from male. In our study, the pork samples were stored during 4 days at 4°C allowing more time to the proteolytic system for action, thus disappearing the differences in hardness among classes. These results agree with those of Bennet et al. (1973)

Table 5
Comparison of sensory descriptors in different quality classes and by sex in pork meat cooked after 4 days of post-mortem storage[a]

	Quality class								Sex			
	PSE ($n=22$)		RSE ($n=7$)		RFN ($n=20$)		DFD ($n=10$)		Male ($n=45$)		Female ($n=14$)	
	M	SE	M	SE	M	SE	M	SE	M	SE	M	SE
Aromatics												
Serum	0.48	0.02	0.46	0.04	0.47	0.02	0.47	0.03	0.44b	0.02	0.50a	0.03
Browned	0.53	0.03	0.53	0.05	0.53	0.03	0.56	0.04	0.53	0.02	0.55	0.04
Pork	4.70	0.08	4.77	0.12	4.70	0.08	4.57	0.11	4.76	0.06	4.61	0.09
Rancid	0.24	0.02	0.21	0.03	0.21	0.02	0.24	0.03	0.22	0.01	0.24	0.02
Boar taint	0.60	0.07	0.51	0.10	0.54	0.06	0.56	0.09	0.60	0.05	0.49	0.08
Strange odors	0.36	0.07	0.30	0.10	0.48	0.07	0.47	0.09	0.48	0.05	0.33	0.08
Tastes												
Bitter	0.45	0.03	0.42	0.04	0.45	0.03	0.40	0.04	0.41	0.02	0.45	0.03
Sour	0.97	0.04	0.90	0.06	0.94	0.04	0.98	0.05	0.87b	0.03	1.02a	0.04
Salty	0.75	0.03	0.73	0.05	0.77	0.03	0.79	0.04	0.72	0.02	0.79	0.04
Sweet	0.43	0.03	0.45	0.04	0.45	0.03	0.49	0.04	0.44	0.02	0.48	0.03
Feeling factors												
Umami	2.32ab	0.08	2.52a	0.13	2.33ab	0.08	2.14b	0.11	2.37	0.06	2.29	0.09
Astringent	0.79	0.04	0.79	0.06	0.78	0.04	0.78	0.05	0.78	0.03	0.79	0.04
Texture												
Juiciness	4.26ab	0.08	4.09b	0.12	4.41a	0.08	4.45a	0.11	4.25	0.06	4.35	0.09
Hardness	3.79	0.08	3.92	0.12	3.81	0.07	3.98	0.10	3.76b	0.05	3.99a	0.09
Overall Quality	5.15	0.09	5.38	0.13	5.18	0.08	5.16	0.11	5.14	0.06	5.28	0.09

[a] Means in a row with different letters are significantly different ($P < 0.05$).

Table 6
Correlation coefficients between composition and texture sensory descriptors with quality

	Juiciness	Hardness	Quality
pH_{2h}	0.144	−0.139	0.104
pH_{24h}	0.339[a]	0.136	−0.194
DL	−0.267[a]	−0.029	0.001
L	−0.259	0.070	−0.268
a	0.307[a]	−0.073	0.503[a]
b	−0.027	−0.08	−0.184
IMF	0.119	−0.103	0.172
Moisture	0.193	−0.006	0.027
Protein	−0.317[a]	0.167	−0.144

[a] Statistical significant difference of the estimated correlations ($P < 0.05$).

who found a highest juiciness in DFD and normal meats but did not find differences in tenderness between DFD and normal meats. On the other hand, Wal, Bolink and Merkus (1988) found a lower hardness in DFD than in normal and PSE beef meats. Other authors found that meat with high post-mortem pH, gave a more juicy and tender meat than the low pH meat (Beltrán et al., 1997; Flores et al., 1999). On the other hand, the overall quality of the cooked pork loin samples was evaluated by the panel taking into consideration all the previous descriptors analysed (aromatics, tastes, feeling sensations, and texture) and it was not evaluated as a preference attribute. The overall quality did not show any significant ($P < 0.05$) difference among quality classes and sex probably due to the long post-mortem storage time (4 days) which contributed to increase the proteolytic activity.

The composition of meat is one of the important factors controlling meat tenderness (Wood et al., 1996). In this study, the juiciness was positively correlated with pH_{24h} and negatively with the protein content and DL (Table 6). On the other hand, the overall quality perceived by the panel was only related with the colour coordinate *a** (Table 6). The correlations among sensory and enzyme activities is shown in Table 7. The rancid aroma was positively related to μ-calpain, m-calpain and RAP. The juiciness was positively related to μ-calpain and m-calpain. The tastes showed several relationships such as the bitter taste that was positively related with DPPI, the sour taste positively related with μ-calpain, m-calpain, AAP and RAP and the salty taste positively related to μ-calpain. The umami sensation, described as savoury and brothy (Maga, 1994), showed a negative relation with μ-calpain, PGAP and DPPII (Table 7).

The data was inspected by PCA in order to reduce the large set of variables and obtain a small number of linear combinations using the following variables: pH_{2h}, pH_{24h}, DL, μ-calpain, AAP_{2h}, $DPPIV_{2h}$, $PGAP_{24h}$, rancid, juiciness, hardness and overall quality. PCA of these variables resulted in three significant factors that accounted for 65% of the variability. The plot of the two first components displayed three main groups of variables along the first (horizontal) axis, with DL being closely related on the left end of PC1 and the enzymes and pH measurements on the right side of the same factor (Fig. 1). The score plot of samples on the PC1–PC2 plane (Fig. 1) stressed the differences between exudative (RSE and PSE) and non-exudative samples (RFN and DFD). The plot of the PC1–PC3 components (Fig. 2) displayed two groups of variables along the vertical axis (PC3), with pH and $PGAP_{24h}$ closely related on the upper part of PC3 and the rancid and pH_{24h} on the lower part of PC3. Also, the score plot of samples on the PC1–PC3 showed the differences between the exudative and non-exudative classes.

Table 7
Correlation coefficients among sensory and muscle enzyme activities at 2 h post-mortem

	μ-Calpain	m-Calpain	Calpastatin	AAP	RAP	LAP	PGAP	DPPI	DPPII	DPPIV
Rancid	0.319[a]	0.332[a]	−0.026	0.133	0.318[a]	0.075	−0.062	0.209	0.118	0.072
Sexual	0.170	0.236	−0.047	0.057	−0.124	−0.134	−0.156	−0.017	0.063	−0.093
Juiciness	0.424[a]	0.357[a]	0.105	0.091	0.185	−0.198	−0.013	0.178	0.028	0.149
Hardness	0.006	0.070	0.171	0.141	0.108	−0.037	0.011	−0.040	0.119	0.190
Bitter	0.214	0.272	−0.138	0.134	0.206	0.007	0.054	0.304[a]	−0.026	0.049
Sour	0.329[a]	0.272[a]	0.036	0.267[a]	0.256[a]	0.017	0.185	0.120	0.186	0.179
Salty	0.307[a]	0.279	−0.014	0.129	0.191	−0.048	−0.031	0.189	0.115	0.139
Sweet	0.046	0.176	0.224	−0.056	0.035	−0.099	0.046	0.024	−0.075	−0.129
Umami	−0.271[a]	−0.221	−0.084	−0.171	−0.113	0.011	−0.264[a]	0.009	−0.335[a]	−0.171
Quality	0.179	−0.011	0.002	−0.182	−0.002	−0.205	−0.076	0.152	−0.227	−0.086

[a] Statistical significant difference of the estimated correlations ($P < 0.05$).

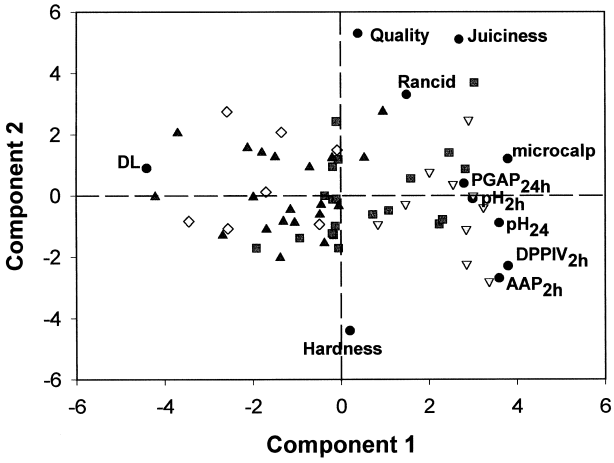

Fig. 1. Principal componentes analysis of technological parameters, enzyme activities and sensory descriptors. Plot of variables on the first two principal components (PC1 and PC2) including the score plot of samples (▲ PSE, ◇ RSE, ■ RFN, ▽ DFD).

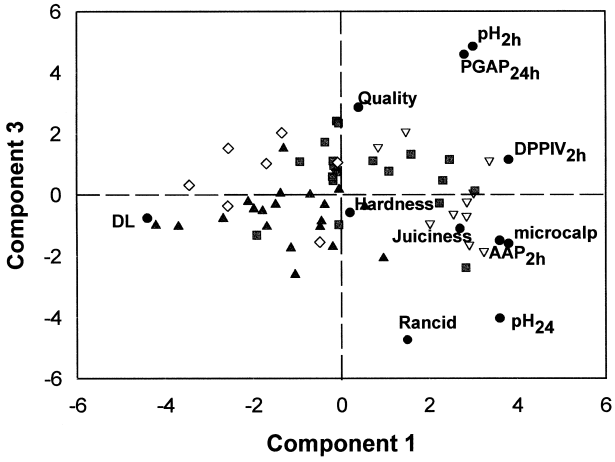

Fig. 2. Principal componentes analysis of technological parameters, enzyme activities and sensory descriptors. Plot of variables on the two principal components (PC1 and PC3) including the score plot of samples (▲ PSE, ◇ RSE, ■ RFN, ▽ DFD).

4. Conclusions

The measure of exoprotease activities can be used for an effective prediction of post-mortem technological pork meat quality. In the case of sensory analysis although some correlations are statistically significant, a practical use of enzyme as predictors would be rather difficult. The assay of aminopeptidase and dipeptidylpeptidase activities (AAP, RAP, LAP, DPPI and DPPIV) at 2 h post-mortem can be used to discriminate between exudative and non-exudative classes. Also, at 24 h post-mortem the differences were detected by the measurement of PGAP, AAP, RAP, DPPII and DPPIV. These measurements are very practical for use in industry because once the industrial kits will be developed, they will be very fast to do it online. The enzyme activity measurements will allow carcass classification on the slaughter-line for a better use of the product for further processing and distribution.

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